Microenvironmental niches in the bone marrow required for B-cell development

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Abstract | B-cell development is known to occur in a complex bone-marrow microenvironment but its functional organization remains unclear. It is thought that bone-marrow stromal cells create distinct microenvironments, known as niches, that provide support for haematopoiesis and B-cell development. Although it has been more than 20 years since the development of a culture system that allows the growth of B-cell progenitors on bone-marrow-derived stromal cells *in vitro*, it is only recently that studies have provided a novel basis for understanding the nature of the niches for B-cell development *in vivo*. This article summarizes the recent advances in research on the earliest B-cell precursors, their requisite environmental factors and the cellular niches that supply these factors and maintain B cells during their development.

Medullary cavities
In typical long bones, such as the femur or the humerus, the shaft consists of a thick walled, hollow cylinder of compact bone with a large central marrow cavity. This cavity is occupied by bone marrow and is called the medullary cavity.

Medullary vascular sinuses
Thin-walled vessels, the endothelium of which allows the passage of large numbers of blood cells. The arterioles in bone marrow continue into the endosteum as typical capillaries that are confluent with medullary vascular sinuses.

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The bone marrow occupies the medullary cavities of bones throughout the skeleton, and haematopoiesis occurs within a complex bone-marrow microenvironment. The bone marrow contains a dense network of medullary vascular sinuses, and blood cells and their precursors are packed in the extravascular spaces between the sinuses[1] (FIG. 1). Because diverse lineages of haematopoietic precursors arise from common multipotential progenitors and develop in the densely cellular spaces in the bone marrow, spatial organization of blood cells and their interaction with the microenvironment within the bone marrow are important for understanding the regulatory mechanisms of haematopoiesis. It has been assumed that the bone marrow contains adherent cells, known as stromal cells, which create microenvironmental niches that maintain blood-cell viability and supply the requisite factors for their development. However, the lack of distinctive characteristics of stromal cells in the bone marrow has made it difficult to identify the niches for various haematopoietic-cell lineages.

It has been previously reported that, during development, haematopoietic cells migrate from the subendosteal region (the inner bone surface) towards the central region of the bone-marrow cavity[2–4]. Consistent with this, it has also been reported by some groups that primitive haematopoietic progenitors are localized in close contact with the endosteum[2,5–8]. By contrast, using different molecular markers to define the progenitors, others have shown that primitive haematopoietic progenitors are uniformly scattered throughout the bone marrow[9,10]. So, it will be difficult to unravel blood-cell behaviour within the bone marrow until specific niches are identified.

B-cell precursors are among the best-characterized haematopoietic precursors, and the extensive study of their development has helped to determine potential pathways for the development of various lineages of blood cells. B cells are generated from haematopoietic stem cells (HSCs) and develop in the bone marrow before they migrate into the blood to reach peripheral lymphoid organs. Of note, end-stage B cells (plasma cells), which develop following the activation of mature B cells by antigen in peripheral lymphoid organs and are crucial for mediating humoral immune responses, return and colonize the bone marrow[11]. Recent findings that are derived mainly from studies using targeted mutation in mice, have determined several environmental factors that are essential for the development and/or retention of the very early B-cell precursors and plasma cells, and have identified several candidates for the cellular niches that these populations inhabit within the bone marrow.

In this Review, I summarize the recent advances in research on early B-cell precursors, their requisite factors and the microenvironmental niches that supply these factors and maintain B cells and their precursors during development in the bone marrow.

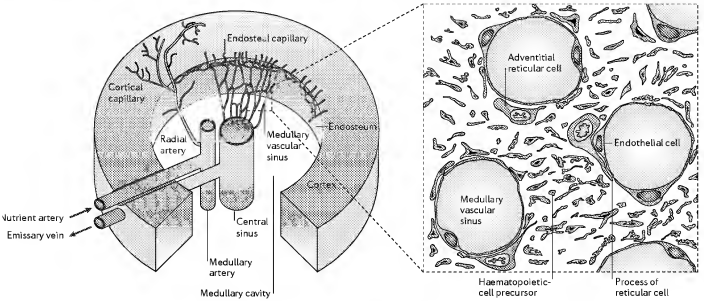

Figure 1 | **Morphological construction of bone marrow.** The bone marrow is contained in the central medullary cavity of bone. The main blood source to the bone marrow is provided by the nutrient artery. The nutrient artery crosses the cortex through the nutrient canal into the medullary cavity, where it divides into ascending and descending arteries, from which radial arteries arise. The radial arteries enter the cortex through the endosteum, which lines the medullary cavity, and become cortical capillaries. Blood from these capillaries can then mix with blood from periosteal capillaries (not shown) and endosteal capillaries. The cortical capillaries enter the medullary vascular sinuses, which form a dense network through the medullary cavity. The sinuses eventually collect and enter the central sinus from which the blood leaves the bone marrow. The medullary vascular sinuses are lined with endothelial cells and surrounded by adventitial reticular cells. Haematopoiesis occurs in the extravascular spaces between the sinuses. (Figure modified from REF. 1.)

Identification of early B-cell precursors

In 1991, B-cell precursors, consisting of cells that are negative for cell-surface immunoglobulin but positive for the B-cell-lineage marker B220, were divided into four subsets according to their differential expression of a range of cell-surface markers during development in the bone marrow. These four subsets were termed fractions A, B, C and D[12] (FIG. 2). Cell-surface phenotypic transition, differences in functional activity and immunoglobulin-gene rearrangement status all support the successive order of these stages from fraction A to B to C to D[12–15]. Because fraction A cells have been shown to progress to fraction B or C phenotypes in *in vitro* stromal-cell culture[12–14], fraction A cells have been suggested to be the earliest identifiable B-cell precursors and have subsequently been termed pre-pro-B cells[12,13]. B cells undergo immunoglobulin-gene rearrangement during their differentiation; B-cell precursors first assemble a heavy-chain DJ (diversity and junction) rearrangement, which is then followed by heavy-chain VDJ rearrangements. Because most pre-pro-B cells lack heavy-chain DJ rearrangements[14], development of the early stages of B-cell precursors is likely to be independent of immunoglobulin-gene rearrangement. Fractions B and C consist mainly of large mitotically active cells, which have heavy-chain DJ or variable (V)DJ rearrangements, and are termed pro-B cells[12,13]. Fraction D consists mainly of small resting cells, termed pre-B cells[12], and immature B cells, which express cell-surface IgM but not IgD, are generated from the cells in fraction D.

Stromal cell
A general term for a large adherent cell. Stromal cells in the bone marrow include reticular cells, endothelial cells and macrophages.

Endosteum
A thin layer of cells that have osteogenic properties and line the medullary cavity

The differentiation potential of pre-pro-B cells. Although cells in the pre-pro-B-cell fraction have been suggested to be the earliest B-cell precursors, several recent studies have shown that the pre-pro-B-cell fraction is heterogeneous and can give rise to haematopoietic lineages other than B cells. For example, a subset of pre-pro-B cells that express pre-T-cell receptor α-chain (pTα) are bipotential precursors of B and T cells[16]. Other subsets in the pre-pro-B-cell fraction can also give rise to other cell types: natural killer (NK) cells can be generated by the NK1.1$^+$ pre-pro-B-cell subset[17], and dendritic cells (DCs), including plasmacytoid DCs, can be generated by both the CD11c$^+$ and Ly6C$^+$ pre-pro-B-cell subsets[18,19]. The observation that the transcription factor paired box protein 5 (PAX5), which induces irreversible B-cell-lineage commitment[20,21], is barely detectable in pre-pro-B cells by real-time quantitative RT-PCR (PCR after reverse transcription of RNA)[9], supports the idea that pre-pro-B cells are not irreversibly committed to the B-cell lineage and retain T-cell-, NK-cell- or DC-lineage potential. In this case, factors produced by specific niches might influence their cell-fate decision. Because *in vitro* experiments might not fully represent their physiological microenvironment, it will be important to know whether the pre-pro-B-cell population *in vivo* contains dominating intermediates in the T-cell-, NK-cell- or DC-lineage pathway.

Intermediates between HSCs and early B-cell precursors. With respect to B-cell development, several issues remain unclear about the developmental pathway from

HSCs to early B-cell precursors, in particular the identity of the intermediate precursor cells. Recent findings using *in vitro* and *in vivo* approaches to evaluate the lineage-differentiation potential of distinct cell populations have indicated that there are several precursor-cell subsets between HSCs and early B-cell precursors that are positive for the B-cell-lineage marker B220 (FIG. 2). Whereas HSCs can be highly purified based on a lineage (Lin)$^-$B220$^-$KIT$^+$SCA1$^+$ fms-related tyrosine kinase 3 (FLT3)$^-$CD34$^-$ phenotype and can give rise to all blood-cell lineages, cells that are Lin$^-$B220$^-$KIT$^+$SCA1$^+$FLT3$^+$CD34$^+$ have been shown to lack erythroid-cell and megakaryocyte potential but retain myeloid-cell and lymphoid-cell potential; these are termed lymphoid-primed multipotent progenitors (LMPPs)[22]. LMPPs might represent a first step towards commitment to the lymphoid-cell lineage. Probably arising from LMPPs is a cell population known as common lymphoid progenitors (CLPs), which are Lin$^-$KIT^{low}SCA1^{low} interleukin-7 receptor (IL-7R)$^+$ or Lin$^-$AA4.1$^+$SCA1^{low}IL-7R$^+$ cells and can generate B- and T-lineage cells but not myeloid-lineage cells[23,24] (FIG. 2). The existence of these multipotent intermediates raises the possibility that their differentiation into B cells is induced by the components of the specific niches.

Furthermore, studies using *in vitro* approaches to evaluate lineage-differentiation potentials have indicated that B220$^-$CD19$^+$ cells can generate myeloid cells and B cells but not T cells[25], and that Lin$^-$KIT^{low}SCA1^{low}IL-7R$^+$FLT3$^+$CD34$^-$ cells or B220$^-$KIT^{low}SCA1$^+$CD24$^+$CD43$^+$ cells (which were independently identified using different markers) are enriched for the early B-cell precursors[26,27], indicating that these populations might be further down the differentiation pathway. Despite these studies, it remains unclear which of these cell populations represent the important intermediates in B-cell development between HSCs and early B-cell precursors. It has been reported that most of the CD4$^-$CD8$^-$CD25$^-$CD44$^{+/-}$KIT^{hi} early T-cell-lineage progenitors (ETPs) that settle in the thymus develop through a LMPP- and CLP-independent pathway, as mice deficient in the transcription factor Ikaros (which

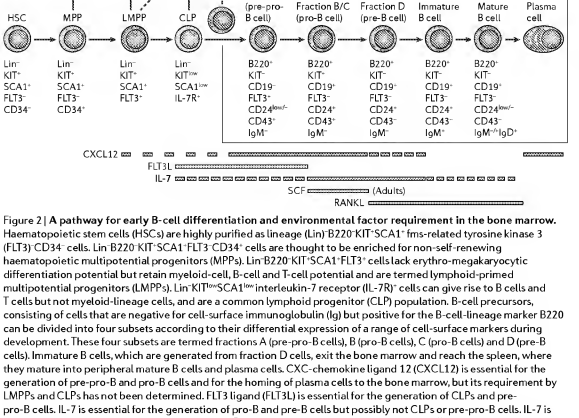

Figure 2 | **A pathway for early B-cell differentiation and environmental factor requirement in the bone marrow.** Haematopoietic stem cells (HSCs) are highly purified as lineage (Lin)$^-$B220$^-$KIT$^+$SCA1$^+$ fms-related tyrosine kinase 3 (FLT3)$^-$CD34$^-$ cells. Lin$^-$B220$^-$KIT$^+$SCA1$^+$FLT3$^-$CD34$^+$ cells are thought to be enriched for non-self-renewing haematopoietic multipotential progenitors (MPPs). Lin$^-$B220$^-$KIT$^+$SCA1$^+$FLT3$^+$ cells lack erythro-megakaryocytic differentiation potential but retain myeloid-cell, B-cell and T-cell potential and are termed lymphoid-primed multipotential progenitors (LMPPs). Lin$^-$KIT^{low}SCA1^{low} interleukin-7 receptor (IL-7R)$^+$ cells can give rise to B cells and T cells but not myeloid-lineage cells, and are a common lymphoid progenitor (CLP) population. B-cell precursors, consisting of cells that are negative for cell-surface immunoglobulin (Ig) but positive for the B-cell-lineage marker B220 can be divided into four subsets according to their differential expression of a range of cell-surface markers during development. These four subsets are termed fractions A (pre-pro-B cells), B (pro-B cells), C (pro-B cells) and D (pre-B cells). Immature B cells, which are generated from fraction D cells, exit the bone marrow and reach the spleen, where they mature into peripheral mature B cells and plasma cells. CXC-chemokine ligand 12 (CXCL12) is essential for the generation of pre-pro-B and pro-B cells and for the homing of plasma cells to the bone marrow, but its requirement by LMPPs and CLPs has not been determined. FLT3 ligand (FLT3L) is essential for the generation of CLPs and pre-pro-B cells. IL-7 is essential for the generation of pro-B and pre-B cells but possibly not CLPs or pre-pro-B cells. IL-7 is required for B-cell differentiation potential of CLPs and pre-pro-B cells. Stem-cell factor (SCF) has an essential role in adults from the pro-B-cell stage. Receptor activator of nuclear factor-κB ligand (RANKL) is involved in the generation of pre-B cells and immature B cells. ETP, early T-cell-lineage progenitor; NK, natural killer.

Ikaros
A haematopoietic-cell-specific member of a family of zinc-finger transcription factors. It is an integral component of a complex containing chromatin remodelling and modifying activities. Ikaros-deficient mice lack all B cells, natural killer cells and fetal T cells, and they have severely reduced numbers of T cells and dendritic cells after birth, indicating that Ikaros is essential for development of lymphoid-lineage cells

is required for the differentiation of lymphoid-lineage cells) have intact ETP populations in the thymus but lack LMPPs and CLPs in the bone marrow[28]. If this is indeed the case, the majority of LMPPs and CLPs might be B-cell precursors in the bone marrow. However, further studies will be needed to address this issue.

Factors essential for B-cell precursors

It is thought that the development of B-cell precursors through these various stages requires the presence of secreted factors in bone-marrow niches, as well as the coordinated expression of specific transcription factors, such as Ikaros, transcription factor E2A, early B-cell factor (EBF) and PAX5. Since Whitlock and Witte first developed a culture system that allows the growth of B-cell precursors on bone-marrow-derived stromal cells *in vitro* in 1982 (REF. 29), several stromal-cell lines that support B-cell development *in vitro* have been established[30–32], and using these cell lines, several microenvironmental components that act on B-cell precursors have been identified. As described below, CXC-chemokine ligand 12 (CXCL12), FLT3 ligand (FLT3L), IL-7, stem-cell factor (SCF) and receptor activator of nuclear factor-κB ligand (RANKL) have each been shown to be essential for B-cell development *in vivo* (TABLE 1).

CXCL12. CXCL12 (also known as SDF1 and PBSF) was the first soluble factor that was reported to be crucial for the earliest stage of B-cell development (pre-pro-B cells)[33]. Chemokines are a large family of structurally related chemoattractive cytokines, which function through heptahelical receptors coupled to heterotrimeric GTP-binding proteins (G-protein-coupled receptors)[34]. CXCL12 was isolated from stromal-cell lines used in *in vitro* B-cell development cultures[35,36] and was first characterized as a growth-stimulating factor for a stromal-cell-dependent B-cell precursor clone[36]. The main physiological receptor for CXCL12 is CXC-chemokine receptor 4 (CXCR4)[36–42], which also functions as an entry receptor for some strains of HIV-1 (REF. 43). Studies using mutant mice with targeted gene disruption have shown that CXCL12 and CXCR4 are essential for various developmental processes in addition to B-cell development, such as angiogenesis and neurogenesis[37,40–42]. Interestingly, it has been shown that CXCL12 is involved in the colonization of bone marrow by haematopoietic cells, including HSCs during ontogeny[37,40,42,44]. Furthermore, it has been reported that CXCR4 is involved in retaining haematopoietic cells in primary lymphoid organs[45]. Consistent with these observations, studies using a transfilter migration assay have shown that various cell types, including haematopoietic precursors, migrate in response to CXCL12 *in vitro*[38,39,46].

In B-cell development, it has been reported that CXCL12-deficient mouse embryos contained severely reduced numbers of $Lin^-CD19^-KIT^+IL\text{-}7R^+AA4.1^+$ cells. These cells are the very early B-cell precursors in fetal liver, which is the main haematopoietic organ during embryogenesis. In addition, the numbers of B-cell precursors, including pre-pro-B cells, are severely decreased in adult bone marrow in chimeric wild-type mice that have been reconstituted with CXCR4-deficient fetal cells, compared with control mice[33]. The CXCL12–CXCR4 axis is therefore essential for the earliest stages of B-cell development in the fetus and adult[33] (FIG. 2). This raises the possibility that CXCL12 attracts and/or tethers precursors immediately after commitment to the B-cell lineage in an appropriate niche. Furthermore, it has been reported that the numbers of pre-B cells are increased, compared with control animals, in the peripheral blood of chimeric mice that have been reconstituted with CXCR4-deficient fetal liver cells or conditional CXCR4-deficient mice in which *Cxcr4* was specifically deleted in B cells from pre-B-cell stage indicating that CXCR4 is involved in retaining pre-B cells in the bone marrow[45,47]. Because the numbers of pre-B cells in the bone marrow of these conditional CXCR4-deficient mice are normal[47], further studies will be needed to understand the contribution of this role of CXCR4 in B-cell development. The CXCL12–CXCR4 axis also has a crucial role in regulating the homing of end-stage B cells to the bone marrow, as CXCR4-deficient plasma cells in the conditional CXCR4-deficient mice or chimeric mice that are reconstituted with CXCR4-deficient fetal liver cells fail to home to the bone marrow[10,48]. Despite these studies, the mechanism by which CXCL12 functions in the regulation of B-cell development remains unclear.

In adult haematopoiesis, studies using chimeric mice that were reconstituted with CXCR4-deficient fetal liver cells have shown that CXCL12 is also involved in the development of T-lineage and myeloid-lineage cells[45,49,50]. However, compared with B-cell development, the effects of CXCL12 on the development of T cells or myeloid cells are much smaller[45,49,50].

FLT3L. FLT3L (also known as FLK2 ligand)[50,52] is a ligand for FLT3 (also known as FLK2)[53,54], which has sequence and structural homology to the class-III-receptor tyrosine kinases that include the macrophage colony-stimulating factor (M-CSF) receptor (c-fms), the SCF receptor (KIT), and the α- and β-platelet-derived growth factor (PDGF) receptors. In FLT3-deficient mice, the numbers of pre-pro-B cells and pro-B cells are reduced by about twofold, whereas numbers of pre-B cells, immature B cells and mature B cells are relatively normal[55]. By contrast, FLT3L-deficient mice have a dramatic reduction in pre-pro-B-cell numbers, slightly reduced numbers of pro-B cells and pre-B cells, and normal numbers of more differentiated B cells, indicating that FLT3L, as well as CXCL12, is essential for the development of pre-pro-B cells[56,57]. Consistent with this, *in vitro* studies have shown that FLT3L stimulates the growth of pre-pro-B cells synergistically with IL-7 (REF. 58). The phenotypic differences between FLT3- and FLT3L-deficient mice might be caused by other as-yet-unknown receptors for FLT3L. FLT3L-deficient mice have normal numbers of $Lin^-KIT^+SCA1^+$ cells, which are highly enriched in various multipotential haematopoietic progenitors, including HSCs[59] and LMPPs[32]. However, they have severely reduced numbers of $Lin^-KIT^{low}SCA1^{low}IL\text{-}7R^+$ CLPs, accompanied by a severe reduction in ETP numbers (TABLE 1), and only a

Transfilter migration assay
An assay that allows the *in vitro* quantification of activities of soluble factors in inducing cell transmigration. It involves a chamber separated by a microporous polycarbonate or nitrocellulose membrane, with the lower compartment for the soluble factor and the upper compartment for the cells. After several hours, the cells harvested from the lower chamber are counted.

Table 1 | **Defects in mice that are deficient in microenvironmental factors in the bone marrow**

Factor	CLPs	B-cell lineage				T-cell lineage			NK cells	DCs	Myeloid cells	Other defects in development	Refs
		Pre-pro-B cells	Pro-B cells	Pre-B cells	IgM$^+$ cells	ETPs	TN2 cells	T cells					
CXCL12	ND	↓↓↓	↓↓↓	ND	ND	ND	↓	↓	ND	ND	↓	Colonization of bone marrow by HSCs; colonization of gonads by PGCs; cardiogenesis; angiogenesis; neurogenesis	33,37, 40,41
IL-7	As WT*	As WT*	↓↓↓	↓↓↓	↓↓↓	ND	↓↓↓	↓↓↓	As WT	As WT	As WT	ND	64,65, 73
FLT3L	↓↓↓	↓↓↓	↓↓	↓	As WT	↓↓↓	↓↓	↓	↓↓	↓↓	↓	ND	55–57
SCF	↓↓	As WT	↓↓↓	↓↓↓	↓↓↓	ND	↓↓↓	↓↓↓	ND	ND	As WT	Erythropoiesis; mast-cell development; gastrointestinal motility; PGC development; melanoblast development	85,87
RANKL	ND	ND	ND	↓↓	↓↓	ND	ND	↓	ND	As WT	As WT	Osteoblast development; lymph-node organogenesis; tooth eruption	92,94

*B-cell differentiation potential was severely impaired. ↓, reduced by <3-fold; ↓↓, reduced by 3–10-fold; ↓↓↓, reduced >10 fold or not detectable. CLP, common lymphoid progenitor; CXCL12, CXC-chemokine ligand 12; DC, dendritic cell; ETP, early T-cell-lineage progenitor; FLT3L, fms-related tyrosine kinase 3 ligand; HSC, haematopoietic stem cell; IL-7, interleukin-7; ND, not determined; NK, natural killer; PGC, primordial germ cell; RANKL, receptor activator of nuclear factor-κB ligand; SCF, stem-cell factor; TN2, triple negative 2; WT, wild-type.

small reduction in more differentiated thymocytes, which supports the idea that FLT3L is crucial for the generation of CLPs[56,60]. However, it is also possible that FLT3L acts on LMPPs, pre-pro-B cells and ETPs. In the development of lineages other than those of lymphoid cells, the studies using FLT3L-deficient mice have shown that FLT3L is also important for the development of conventional and plasmacytoid DCs[61,62], and NK cells[61]. Interestingly, these lineage cells have been reported to arise from the cells in the CLP or pre-pro-B-cell fraction, as mentioned earlier[17–19].

IL-7. IL-7 was isolated on the basis of its ability to induce pro-B-cell proliferation[63] and is the first cytokine that has been shown to be essential for lymphoid development[64,65]. The receptor for IL-7 is comprised of two chains: IL-7Rα and the cytokine-receptor common γ-chain (γ_c), which as its name suggests, is shared among the receptors for IL-2, IL-4, IL-9 and IL-15, as well as IL-7. A member of the Janus kinase (JAK) family of intracellular tyrosine kinases, JAK3, associates constitutively with γ_c and is a downstream effector of the γ_c signalling pathway, as indicated by the similar phenotypes observed in γ_c-deficient and JAK3-deficient mice[66–70].

IL-7- and IL-7Rα-deficient mice have severe defects in the development of B and T cells (including the γδ T-cell subset) but not NK- or DC- lineage cells[64,65,71,72]. In B-cell development, analysis of IL-7- or IL-7Rα-deficient mice has shown that the bone marrow of these mutant mice contains normal numbers of pre-pro-B cells but severely reduced numbers of pro-B cells, pre-B cells and the more differentiated B cells, indicating that IL-7 has a crucial role at the pro-B-cell stage[64,65]. Consistent with this, IL-7 has marked activity in inducing the proliferation of pro-B cells, but not pre-pro-B cells, *in vitro*[12]. However, in contrast to these observations, a recent analysis of IL-7-deficient mice and an *in vitro* evaluation of the lineage-differentiation potentials has shown that whereas normal numbers of CLPs and pre-pro-B cells were detected, the B-cell differentiation potential of these cells was severely impaired, resulting in the observed severe reduction of pro-B-cell numbers[73]. Furthermore, this recent study showed that Lin$^-$KIT$^+$SCA1$^+$ multipotential haematopoietic cells from IL-7-deficient mice are functionally identical to control cells in their ability to generate B cells and myeloid cells in an *in vitro* culture system[73]. Based on these results, it seems likely that IL-7 functions at the CLP stage of B-cell development[73].

The mechanisms by which IL-7 functions in B-cell development are well characterized. The stages at which B-cell development is impaired in mice with B-cell-targeted deletion of myeloid-cell leukaemia sequence 1 (MCL1), an anti-apoptotic B-cell lymphoma 2 (BCL-2)-family member, are similar to the defects observed in IL-7- or IL-7Rα-deficient mice, which supports the idea that IL-7 induces the expression of MCL1 to mediate the survival of B-cell precursors[74]. Furthermore, IL-7Rα has been shown to deliver a signal that specifically induces the recombination of D-heavy-chain to distal V-heavy-chain segments in pro-B cells, by altering the

accessibility of DNA substrates to the recombinase[75]. And, it has been reported that induced overexpression of the transcription factor EBF, which is essential for B-cell differentiation at a stage before pro-B cells[76], is sufficient to restore the defects in the B-cell differentiation potential of IL-7-deficient CLPs or IL-7Rα-deficient HSCs[73,77], indicating that IL-7 has a role in upregulating EBF activity to maintain the B-cell differentiation programme. Although IL-7Rα-deficient mice lack pro-B cells, IL-7-deficient mice contain some pro-B cells, indicating that this difference might be a result of other cytokines that use IL-7Rα[64,65]. Thymic stromal lymphopoietin (TSLP), the receptor of which consists of a heterodimer of IL-7Rα and another receptor subunit, is a candidate cytokine[78], but studies using TSLP-receptor-deficient mice have indicated that TSLP is not essential for B-cell development *in vivo*[79,80]. Further analyses of mice that are deficient in both a candidate cytokine and IL-7 expression will be needed to address this issue.

SCF. SCF is a ligand for the class-III-receptor tyrosine kinase KIT and is encoded by a gene that maps to the steel (*Sl*) locus of mice[81–83]. Naturally occurring mutant mice that lack SCF (*Sl/Sl* mice) and KIT (W/W mice) have similar phenotypes, with developmental blocks in many cell types. This indicates an important role for the SCF–KIT axis in the development of many cell types, including primordial germ cells, melanoblasts and haematopoietic cells such as multipotential progenitors, erythrocytes, mast cells and T cells[59,84,85] (TABLE 1). All stages of B-cell development in W/W mouse embryos and neonates were phenotypically indistinguishable from those in wild-type mice, indicating that SCF is not required for B-cell development *per se*[85]. Therefore, the reduced number of B-cell precursors in W/W mice is thought to be caused at the level of multipotential progenitors. Paradoxically, many studies have shown that SCF has marked activity in inducing the *in vitro* proliferation of pro-B cells synergistically with IL-7 (REFS 81,86). However, because studies on the function of SCF in adults were precluded by the fact that W/W and Sl/Sl mice die around the first week of life from anaemia (that is, reduced erythrocyte numbers), these *in vitro* and *in vivo* studies are not directly comparable. Recently, viable W/W mice, termed the Vickid (viable c-KIT deficient) and Wepo/W mutant mice rescued by erythropoietin mice, which contain approximately two-fold more erythrocytes than W/W mice, have been described, and the role of KIT in B-cell development in young and adult mice was investigated[87]. In Vickid mice younger than 4 weeks of age, B-cell development seemed normal, although the numbers of $Lin^-AA4.1^+SCA1^{low}IL\text{-}7R^-$ CLPs were severely reduced[87]. However, the numbers of pro-B cells decreased as Vickid mice aged. At 195 days (approximately 28 weeks), the numbers of pre-pro-B cells were normal but the numbers of pro-B and pre-B cells were severely reduced in the mutant mice[87]. Similar phenotypes were found in B-cell development in Wepo mice[87]. Together, these results indicate that the SCF–KIT axis is redundant in B-cell development in fetal, neonatal and young mice, but that it has an essential role in adult mice. The studies using naturally occurring mutant mice in which sequences encoding the transmembrane domain of SCF have been eliminated, show that a membrane-bound isoform of SCF is required for haematopoiesis[88] and might have a role in B-cell development. This is consistent with the idea that cells expressing membrane-bound SCF might function as a niche for pro-B cells and pre-B cells[89].

RANKL. The transmembrane protein RANKL (also known as OPGL and ODF) is a tumour-necrosis-factor (TNF) family member that is essential for the development of osteoclasts and for bone remodelling[90–92]. The receptor for RANKL is the TNF-receptor (TNFR)-family member RANK, which is expressed by DCs, T cells and osteoclast precursors[93,94]. Studies using mice with disrupted *Rankl* or *Rank* genes have shown that the RANK–RANKL axis is essential for lymph-node organogenesis and is involved in the development of B and T cells but not DCs[92,94]. Reconstitution of immunodeficient recombination-activating gene 1 ($Rag1^{-/-}$) mice with $Rankl^{-/-}$ fetal liver cells led to reduced numbers of pre-B cells and $B220^+IgM^+$ immature B cells, whereas reconstitution of $Rankl^{-/-}$ mice with wild-type bone-marrow cells led to normal B-cell development, indicating that RANKL expression by lymphoid cells is important for B-cell development[92]. It has been reported that RANKL is highly expressed by bone-marrow-derived primary stromal cells and osteoblasts[90]. It will be important to know which cells produce RANKL and RANK, and which cells they act on in B-cell development.

Cells in niches for B-cell development

All the factors required for B-cell development that are discussed here are thought to be produced by the cells that are located in the bone marrow and must be delivered to the appropriate B-cell precursor at the appropriate stage of development. But where precisely in the bone marrow do B cells and their precursors develop? Furthermore, how do specific microenvironments support the development of B cells through the ordered series of stages? To address these issues, it is important to know which cells in the bone marrow can create the specific microenvironmental niches that maintain B cells and supply the requisite factors for their development and have a regulatory potential that persists even when blood cells are absent. Ultrastructural studies indicate that reticular cells, which surround bone-marrow sinuses as adventitial reticular cells and branch into the surrounding haematopoietic space, are the most abundant type of stromal cell in the bone marrow, with which B cells have been shown to be associated, making these adventitial reticular cells likely candidates[4,4,95]. However, evidence that these adventitial reticular cells function as a niche for haematopoiesis has been difficult to obtain. And, although distinctive stromal-cell lines have been shown to produce different environmental factors *in vitro*[96,97], it is unclear whether a single type or more than one type of stromal cell is involved in the niche

***Sl/Sl* mice**
Mice homozygous for a spontaneous null mutation at the steel (*Sl*) locus located on chromosome 10 die around the first week of life, with many defects including severe anaemia, lack of hair pigmentation, sterility and defective intestinal pacemaker function. They have deficiencies in melanoblasts, primordial germ cells and haematopoietic cells, including multipotential progenitors, erythrocytes and mast cells.

W/W mice
Mice homozygous for a spontaneous null mutation at the dominant white spotting (W) locus located on chromosome 5 die around the first week of life, with the same symptoms as Sl/Sl mice.

Vickid mice
(Viable c-KIT-deficient mice). Vickid mice are white, black-eyed, apparently healthy KIT-deficient mice that arose from a cross between W/+ and W/+ heterozygous mice. The molecular basis for the survival of these mice is unknown.

Wepo mice
(W mutant mice rescued by erythropoietin). Wepo mice were generated by crosses between erythropoietin transgenic W/+ mice and W/+ mice. Transgenic expression of erythropoietin is thought to boost erythropoiesis even in the absence of KIT, and rescue the W/W mice.

Osteoclasts
Multinucleated cells that resorb bone. They are specialized monocyte/macrophage family members that arise from haematopoietic stem cells. Morphogenesis and remodelling of bone is controlled by processes that include the synthesis of bone by osteoblasts and the coordinate resorption of bone by osteoclasts.

Reticular cells
The main stromal cells in the bone marrow, with dendritic-cell (DC)-like morphology. Unlike DCs, they have broad, sheet-like cytoplasmic processes.

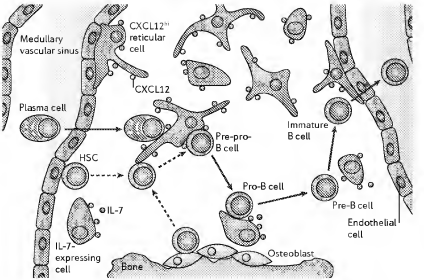

Figure 3 | Candidates for cellular niches for B-cell development and a model of the movement of B cells and their precursors in the bone marrow. In this model, the intermediate precursor cells between haematopoeitic stem cells (HSCs) — which are located near the osteoblasts[7,8], endothelial cells[113] or CXC-chemokine ligand 12[hi] (CXCL12[hi]) reticular cells[10] — and pre-pro-B cells would move towards CXCL12[hi] reticular cells. Pre-pro-B cells associate with CXCL12[hi] reticular cells, whereas pro-B cells move away and instead adjoin interleukin-7 (IL-7)-expressing cells[10]. Subsequently, pre-B cells leave IL-7-expressing cells[10]. B cells expressing cell-surface IgM exit the bone marrow and enter the blood to reach the spleen, where they mature into peripheral mature B cells. End-stage B cells (plasma cells) again home to CXCL12[hi] reticular cells in the bone marrow[10].

function *in vivo*. Recently, several cell types have been implicated in providing the specific cellular niches for B-cell development (FIG. 3), as described next.

Osteoblasts. Osteoblasts are highly specialized cells that are responsible for synthesis, deposition and mineralization of the extracellular matrix of bone. They are thought to arise from mesenchymal stem cells (MSCs) and have an important role in the development of all bones. Both *in vitro* and *in vivo* studies indicate that osteoblasts are also essential regulators of the development of all blood cells, including B cells in the bone marrow, and such studies indicate that osteoblasts might function as niches for HSCs[7,8,98–100]. In agreement with these observations, a recent study has shown that primary mouse osteoblasts can support early B-cell lymphopoiesis in *in vitro* cultures[101]. Furthermore, the observations that human osteoblasts express CXCL12 (REF. 102) and that primary mouse osteoblasts express RANKL[84], indicate that osteoblasts might participate in B-cell lymphopoiesis.

CXCL12[hi] reticular cells. The observation that CXCL12 is required for the generation of the earliest identifiable B-cell precursors, pre-pro-B cells[33], indicates that CXCL12 might attract and/or tether these precursors immediately after commitment to the B-cell lineage in an appropriate microenvironment for their development. Therefore, the localization of cells expressing CXCL12 in the bone marrow has been analysed using mice in which the gene encoding green fluorescent protein (GFP) was knocked into the *Cxcl12* locus (*Cxcl12–GFP* knock-in mice)[10]. High levels of expression of CXCL12–GFP was seen in a small population of stromal cells (termed CXCL12[hi] reticular cells). Lower levels of CXCL12–GFP expression were found in some other fibroblast-like cells. CXCL12[hi] reticular cells occurred singly and were uniformly scattered throughout the bone marrow[10]. Within the bone marrow, reticular cells as well as sinusoid endothelial cells have been shown to express vascular cell-adhesion molecule 1 (VCAM1)[103]. Almost all of the CXCL12[hi] reticular cells expressed VCAM1, and constituted ~20% of the total VCAM1[+] cells[10], supporting the idea that CXCL12[hi] reticular cells are a subset of bone-marrow reticular cells. In addition, almost all of the CXCL12[hi] reticular cells lacked expression of platelet/endothelial cell-adhesion molecule 1 (PECAM1) and phenotypic markers of osteoblasts, including osteopontin and osteocalcin, indicating that CXCL12[hi] reticular cells are different from endothelial cells and osteoblasts. Consistent with this observation, CXCL12[hi] reticular cells are located away from the bone surface. The molecular mechanisms that control CXCL12 expression in CXCL12[hi] reticular cells remain unclear. It has however been shown that the cytokine granulocyte colony-stimulating factor (G-CSF) induces a reduction of CXCL12 expression in the bone marrow[104,105]. Further studies investigating the effect of environmental factors, including G-CSF, on CXCL12[hi] reticular cells are needed.

IL-7-expressing cells. Cells that express IL-7 in the bone marrow have been examined by immunohistochemical analysis. Staining with an IL-7-specific antibody was observed for some fibroblast-like cells, which also expressed VCAM1 (REF. 10). These IL-7-expressing cells were scattered throughout bone marrow[40,106]. Because both CXCL12 and IL-7 are required for B-cell lymphopoiesis and they have synergistic or additive functions on B-cell precursors, expression of both these factors by stromal cells would be expected[33,36,37,65]. However, analysis of bone marrow from *Cxcl12–GFP* knock-in mice showed that CXCL12[hi] reticular cells did not stain with IL-7-specific antibody and that the IL-7-expressing cells were located away from the CXCL12[hi] reticular cells[10], indicating that these cells might represent distinct stromal-cell subsets. It has previously been shown that IL-7 expression by a stromal-cell line is induced by stimuli, including several cytokines, and B-cell precursors[107]. How IL-7 expression is regulated in cellular niches comprised of IL-7-expressing cells, for example, is an important issue for the future.

B-cell-precursor–niche interactions

To clarify the functions of niches for B-cell development, it is important to characterize the association of these niches with B cells and their progenitors. To achieve this, the association between multipotential haematopoietic progenitors and CXCL12[hi] reticular cells was analysed[10]. Immunohistochemical staining analysis using antibodies against KIT and SCA1 was carried out to visualize multipotential haematopoietic progenitors, including

HSCs and LMPPs[108]. KIT$^+$SCA1$^+$ cells were detected throughout the bone marrow and were mostly in contact with the processes of CXCL12^{hi} reticular cells. It has been suggested that CXCL12^{hi} reticular cells that are located adjacent to endothelial cells might function as a HSC niche in fetal bone marrow[44]; it is therefore possible that HSCs remain adjacent to CXCL12^{hi} reticular cells in adult bone marrow. By contrast, recent studies indicate that HSCs are located adjacent to the osteoblast cells that line the bone surface[7,8]. In this model, intermediates between HSCs and B-cell precursors would move from the bone surface towards the processes of CXCL12^{hi} reticular cells (FIG. 3).

Because cell-surface expression of FLT3 is restricted, within the B220$^+$ B-cell population, to the pre-pro-B-cell fraction[40], this cell fraction was used as a marker to track the location of pre-pro-B cells in the bone marrow. Most B220$^+$FLT3$^+$ cells were shown to be in contact with CXCL12^{hi} reticular cells. By contrast, most B220$^+$KIT$^+$ pro-B cells (FIG. 3) were located away from the CXCL12^{hi} reticular cells and instead associated with the IL-7-expressing cells. Furthermore, most B220$^+$IL-7Rα$^+$ cells, consisting mainly of pre-B cells[15], were found not to be in contact with either CXCL12^{hi} reticular cells or IL-7-expressing cells, implying that the cells that differentiate into pre-B cells leave IL-7-expressing cells. Furthermore, most B220$^+$IgM$^-$ cells, comprising newly generated immature B cells and mature B cells, were not in contact with CXCL12^{hi} reticular cells or IL-7-expressing cells. The B cells that express cell-surface IgM exit the bone marrow and enter the blood to reach the spleen, where they mature into peripheral mature B cells. Following their encounter with antigen in the periphery, the mature B cells differentiate into long-lived plasma cells, which return to the bone marrow. As bone-marrow plasma cells require CXCR4 for their homing and/or retention in the bone marrow[10,48], the observation that plasma cells associate with CXCL12^{hi} reticular cells within bone marrow was not surprising[10]. In fact, by using antibodies that are specific for IgG and the plasma-cell marker syndecan-1 (REFS 109,110), almost all of the IgG$^+$ syndecan-1-expressing plasma cells were shown to be in contact with the processes or bodies of CXCL12^{hi} reticular cells[10].

In summary, because CXCL12^{hi} reticular cells represent a small population of reticular cells, they might constitute a specific niche for CXCL12-dependent cells at the earliest and end stages of B-cell development[10]. The observation that most KIT$^+$SCA1$^+$ multipotential haematopoietic progenitor cells adhere to the CXCL12^{hi} reticular cells supports the idea that commitment to the B-cell lineage occurs adjacent to CXCL12^{hi} reticular cells. Of note, it is possible that CXCL12^{hi} reticular cells also function as a niche for precursors of other lineages, including DC and NK-cell precursors, which might be included in the pre-pro-B-cell fraction. Furthermore, the IL-7-expressing cells are likely to provide the niches that induce clonal expansion of B-cell precursors. Because IL-7 can diffuse in bone-marrow mesenchyme and acts on CLPs and pre-pro-B cells[73], IL-7-expressing cells might also have a role in the earliest stages of B-cell development. It has been shown that most of the cells that have differentiated into pre-B cells were not in contact with CXCL12^{hi} reticular cells or IL-7-expressing cells. This is consistent with the idea that late pro-B cells that express the pre-B-cell receptor have a lowered threshold for proliferation in response to IL-7 (REF. 111) and that pre-B cells become independent of the stromal microenvironment[12,111].

The mechanisms that regulate the association between developing B cells and their cellular niches remain unclear, as do the exact role(s) of the microenvironmental factors. CXCL12, for example, has several possible roles: it might attract cells to the niche, it might enhance their ability to adhere to a particular niche (possibly through activation of integrins) and/or it might support their survival within the niche[10,33].

Future directions

Approximately 20 years after Whitlock and Witte developed the culture system that allowed B-cell progenitors to be grown on bone-marrow-derived stromal cells *in vitro*[29], we have finally begun to provide a novel basis for understanding the nature of the microenvironmental niches for B-cell development *in vivo*. Nevertheless, a central question remains: why do specific cellular niches for B-cell development exist within the bone marrow? It is possible that specific cellular niches supply the appropriate combinations of microenvironmental factors that are required for the differentiation, survival or proliferation of B cells and their precursors. For example, a certain combination of cytokines that induce the expansion of B-cell precursors might prevent their further differentiation. By contrast, it has been shown that stimulation within the thymic environment, or *in vitro* with several cytokines, induces the B-cell precursors in mice lacking PAX5 to differentiate into T-cell, NK-cell or myeloid-cell lineages[20,21]. Given that *Pax5* mRNA can be detected from the pro-B-cell stage[10], the earlier B-cell precursors would still be expected to be responsive to lineage-inappropriate signals. This indicates that B-cell precursors might be protected from lineage-inappropriate signals in the specific niches.

Our increasing knowledge of lineage potential and phenotype of cells between HSCs and early B-cell precursors will no doubt help us to further characterize the interaction between important precursor populations and the candidate cells of bone-marrow niches for B-cell development, including osteoblasts, CXCL12^{hi} reticular cells and IL-7-expressing cells. However, further studies are required to identify the cells that supply FLT3L, SCF, RANKL or other unknown requisite factors for B-cell development. Furthermore, the roles of other types of stromal cell, including endothelial cells, which have recently been shown to function as niches for HSCs[113] and megakaryocytes[114] in B-cell development, will be the subject of future research. Such studies will provide an insight into the nature of spatiotemporal regulation of B-cell development *in vivo* and address the fundamental question of how diverse lineages of haematopoietic precursors arise from HSCs and develop in densely cellular extravascular spaces within the bone marrow.

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Competing interests statement

The author declares no competing financial interests.

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